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Abstract

Age of *Penaeus vannamei* influences infections with *Baculovirus penaei* in different ways. The virus has less effect on host survival in the older of seven tested age-groups of shrimp experimentally administered virus; no virus-induced mortality occurred in postlarvae older than 63 days. Prevalence of newly acquired infections decreases as age increases, sometimes to zero. Prepatency periods appear to increase by a few days in infections in most successively older tested age-groups. Actual and relative numbers of affected hepatopancreatic cells decrease from 75 or 80% typically seen in postlarvae infected when 3 days old to ≤ 5% typically seen in infected individuals exposed when they were 63 to 157 days old; infections become undetectable in individuals exposed when they were 325 and 454 days old.

Introduction

The virus *Baculovirus penaei* (BP) infects the hepatopancreas (HP) and anterior midgut of penaeid shrimps. Assessing data on susceptibility of shrimp to this virus relative to host age is important to gain an understanding of infections. For larvae of *Penaeus vannamei* and a few other penaeid shrimps, losses approaching 100% of affected stocks are common, whereas infections in juveniles may be subacute to chronic (Lightner, 1988; Overstreet et al., 1988).

Information on the susceptibility to BP by penaeids is limited. Experimental infections of virus-free laboratory-reared third substage protozoeal through early postlarval stages are possible. Overstreet et al. (1988) developed a bioassay with *P. vannamei* that usually produces 100% infection in all those stages. Previously, experimental transmission of BP to juvenile and adult shrimp had been minimally successful (Couch, 1974, 1976, 1978). Moreover, some of Couch's studies involved wild shrimp, some of which may have had latent infections. In a survey of BP in wild adult *P. duorarum* in the same area in Florida, Couch (1976) reported an average natural prevalence of 20%, including light to heavy infections.

The object of this study was to assess effects of age on susceptibility of a penaeid to BP. Resulting information provides a more complete understanding of BP transmission and a basis for developing strategies relevant to transfer, sales, stocking, and disinfection of penaeids, especially *P. vannamei*.

Materials and Methods

The virus used was a highly virulent BP strain from infected *P. vannamei* larvae or postlarvae derived from experimental infections performed at the Gulf Coast Research Laboratory (Overstreet et al., 1988). Approximately 1,200 of these protozoeal larvae, determined to be infected with BP polyhedra (also called PIBs, or occlusion bodies) by microscopic observation of the HP, were homogenized in 3 ml distilled water with a Dounce tissue grinder. This homogenate was fed over a 2-h period to approximately 20,000 24- to 36-h-old nauplii of the brine shrimp, *Artemia franciscana* (previously *A. salina*). The nauplii and the residual uneaten virus-containing material were divided into six equal portions (approximately 3,000 brine shrimp per portion) and one portion offered to each of the different age-groups of *P. vannamei* except a 120-day group. Each shrimp group was held in a 38-l glass aquarium containing 20 l of 32 ppt salt water produced from hw-Marinemix® with bioelements (Hawaiian marine mix). Water was never exchanged; water quality was maintained with constant aeration, biological sponge filters, and Fritzyme®. Air cooling and heating units were used to maintain water temperature in an insulated room. Water temperature, ammonia concentration, and nitrite concentration were monitored on a daily basis. The diet for the shrimp throughout the experiment consisted of 24- to 48-h brine shrimp. Negative controls for each of the seven age-groups were maintained in the same manner as described above, except during the first week of the experiment when those shrimp were fed a commercial shrimp diet.

Stocking densities of *P. vannamei* varied according to the number of individuals of each age-group available. Groups of shrimp aged 3, 39, and 63 days (based on time after molting into postlarvae) were stocked at a rate of 70 to 80 per 20 l of water. Older groups of 120-, 157-, 325-, and 454-day individuals were stocked at rates of 40 (in each of two aquaria), 30, 14, and 5 per 20 l of water, respectively. All tests exposing brine shrimp contaminated with virus to the groups were conducted concurrently except the test with the 120-day group, and that was started 4 weeks later. Concurrent controls for each group were stocked at the same densities as their experimental counterparts.

The sampling schedule was determined on the basis of our experience with infected shrimp, a preliminary test conducted with a group of shrimp initially 56 days old, and the limited availability of laboratory-reared, virus-free individuals in age-groups pertinent for this study. Consequently, to conserve those limited specimens, small or no samples were taken during the early time periods when extrapolation of findings from larval and preliminary tests suggested that most values would be negative.

The entire fresh HP was used to diagnose polyhedra. It was removed using the procedures described by Overstreet et al. (1988). For all age-groups except the youngest, three random subsamples of each HP were placed in saline on a regular microscope slide covered gently with a coverglass and examined for the presence of polyhedra using light microscopy. The remaining HP-material was fixed in Davidson's fixative so the diagnosis made on the basis of fresh material could later be confirmed with hematoxylin and eosin-stained (H&E) paraffin sections. Since the HP was small in shrimp of the youngest group, the entire HP was examined fresh on a regular microscope slide. For corroboration of those infections, several additional randomly selected whole larvae were fixed and sectioned.

Results

Subsamples of three or four individuals from the group initially 56 days old in the preliminary test exhibited the following values for prevalence of infection at the indicated days after being fed virus: 0% at day 4; 100% at day 7; 33% at days 14, 21, 31, 40, 52, and 66; and 0% at days 80 and 87. These data fit with but show a higher prevalence than in the primary tests.

The primary tests indicated a difference in the susceptibility to patent BP infections by different age-groups of *P. vannamei* (Table 1). Viral-fed shrimp initially aged 3, 39, 63, 120, and 157 days exhibited maximum prevalence values of 100, 30, 42, 10, and 20%, respectively. In groups (except for all those in the preliminary test) examined from 25 to 60 days, no polyhedra were observed (Table 1). Older shrimp exposed when they were 325 and 454 days old showed no signs of diagnostic hypertrophied nuclei or polyhedra. Prepatency periods appeared to be longer in older shrimp (Table 1). Members of the three younger shrimp groups tended to have more intense infections than those of the older groups in the number of cells infected and the number of polyhedra per infected cell. Estimates of the percentage of HP-cells excluding those at the tips of the tubules reached 80, 60, 30, 0.5, 35 (one with 80), 0, and 0% of HP-cells in groups exposed at 3, 39, 63, 120, 157, 325, and 454 days, respectively. Typically, fewer than 1% of the HP-cells in shrimp older than those in the 63-day group had an infection. HP-cells in individuals of young groups typically contained six to ten polyhedra whereas those of older group members usually but not always contained fewer than six.

Table 1. Prevalence of BP infections in *Penaeus vannamei* exposed at different ages

Days post feeding	Age of shrimp after reaching postlarva (days) 3				39				63			
	BP[1]		C[2]		BP		C		BP		C	
	N[4]	%[5]	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
4	5	(0)	—	—	4	(0)	—	—	—	—	—	—
5	21	(77)	17	(0)	4	(0)	—	—	—	—	—	—
9	20	(100)	20	(0)	7	(14)	7	(0)	—	—	—	—
10	—	—	—	—	20	(20)	20	(0)	20	(20)	20	(0)
14	*	*	—	—	20	(30)	10	(0)	21	(42)	19	(0)
16					—	—	—	—	*	*	—	—
17					—	—	—	—				
19					—	—	—	—				
25					—	—	—	—				
30					—	—	—	—				
40					—	—	—	—				
60					20	0	—	—				
Mean TL (cm)	0.6		0.6		1.5		1.5		2.8		2.8	
Measurement conducted	Day 9				Day 14				Day 14			

Continued

120[3]				157				325				454			
BP[1]		C[2]		BP		C		BP		C		BP		C	
N[4]	%[5]	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	(0)	10	(0)	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	(0)	10	(0)	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	(0)	10	(0)	20	(20)	12	(0)	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	14	(0)	11	(0)	—	—	—	—
10	(10)	10	(0)	—	—	—	—					5	(0)	5	(0)
10	(0)	10	(0)	—	—	—	—								
10	(0)	10	(0)	—	—	—	—								
10	(0)	10	(0)	—	—	—	—								
—	—	—	—	6	(0)	—	—								
4.0		4.1		3.5		4.4		6.9		8.2		15.1		15.0	
Day 19				Day 16				Day 17				Day 19			

[1]Fed BP; [2]control shrimp not fed or exposed virus; [3]test conducted 4 weeks after others; [4]sample size; [5]prevalence of BP infection in percent. *No sample available because of mortalities; —, samples available but not sampled

All diagnoses based on fresh material were confirmed by H&E paraffin staining. Staining a portion of selected fresh material with aqueous 1% solutions of either phloxin B followed by fluorescence microscopical examination or malachite green as recommended by D.V. Lightner (personal communication, 1988) made some observations easier but did not change the results.

Water quality remained constant throughout the experiment. Ammonia and nitrite levels were ≤ 0.1 ppm and temperature was 26 ± 1°C.

Discussion

Tabulated data suggest that among individuals of *Penaeus vannamei*, older shrimp are less susceptible to patent BP infections than younger ones. Overstreet et al. (1988) demonstrated the vulnerability of larval and early postlarval shrimp to experimental infections and indicated difficulty in infecting older individuals. Our study corroborated those findings and suggested that infections in older shrimp were less extensive and did not persist. Couch (1976) also supported this less extensive aspect of infections. He determined a natural 20% prevalence in 2000 specimens of the pink shrimp, *P. duorarum*, collected by bait dealers in northwestern Florida. Couch (1978) also observed infections in about 20% of both hatchery-reared and wild juvenile or adult pink shrimp 20 to 30 days after being fed heavily infected HP.

Even though limited by the relatively small number of older shrimp used, this study suggests that as shrimp age, they become less vulnerable to new and existing infections. Mortalities reaching 100% of the unsampled shrimp exposed when 3 and 63 days old took 14 and 16 days, respectively. Detecting polyhedra in the rapidly decaying HP of those dead individuals was difficult and sometimes impossible. Nevertheless, based on observations of larval death resulting from BP by Overstreet et al. (1988), low level of mortality in controls, and normal daily nitrite and ammonia levels, most observed mortalities probably resulted from or at least were facilitated by BP infection. Even though fed the same source of BP as the young shrimp, shrimp initially aged 120 days older exhibited few deaths, further indicating their increased resistance to the BP disease.

Prepatent and patent periods also exhibited variation in BP infections associated with shrimp age. Overstreet et al. (1988) demonstrated prepatent periods of 1 and 2 days in larval shrimp. In this experiment, patent infections developed by the fifth day in young postlarvae, by a few days longer in specimens over 1 month old, and perhaps never in most individuals over a half year old, as indicated by the lack of hypertrophied nuclei in our test animals. This resistance with age is probably related to the loss of patent infections as also exhibited during the preliminary study.

The times at which patent infections were first observed and the times at which prevalence was maximal may not exhibit a perfectly linear relationship; however, those times for the different age-groups did exhibit an obvious trend. The absence of samples at about 30 and 60 days for shrimp initially fed at 325 and 454 days, respectively, constitutes an important void.

A variety of factors could affect the prepatent period and other age-related differences. Our unpublished studies with larvae suggest that some of those factors involve genetic

makeup and nutrition of the parents as well as nutrition of and stress on the larvae. Also, Bell and Lightner (1987) noted some trends associating IHHN virus infections with age of *P. stylirostris*.

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